

What is claimed is:

1. A mutant Green Fluorescent Protein (GFP) from *Renilla reniformis*, wherein the mutation comprises an amino acid substitution in the Beta Strand 4 portion of the protein, relative to the wild-type form of the protein, and wherein the mutant GFP protein has one or more of the following characteristics:
 - (a) enhanced emission intensity, relative to wild-type GFP protein from *Renilla reniformis*;
 - (b) a narrower or broader emission spectrum, relative to wild-type GFP protein from *Renilla reniformis*;
 - (c) a shift in excitation or emission maxima, relative to wild-type GFP protein from *Renilla reniformis*;
 - (d) a shift in maturation rate, relative to wild-type GFP protein from *Renilla reniformis*; and
 - (e) exhibiting less quenching of fluorescence at acidic pH, relative to wild-type GFP protein from *Renilla reniformis*.
2. A mutant Green Fluorescent Protein (GFP) from *Renilla reniformis*, wherein the mutation comprises an amino acid substitution in the loop region of the protein between Beta Strand 2 and Beta Strand 3, relative to the wild-type form of the protein, and wherein the mutant GFP protein has one or more of the following characteristics:
 - (a) enhanced emission intensity, relative to wild-type GFP protein from *Renilla reniformis*;
 - (b) a narrower or broader emission spectrum, relative to wild-type GFP protein from *Renilla reniformis*;
 - (c) a shift in excitation or emission maxima, relative to wild-type GFP protein from *Renilla reniformis*;
 - (d) a shift in maturation rate, relative to wild-type GFP protein from *Renilla reniformis*; and
 - (e) exhibiting less quenching of fluorescence at acidic pH, relative to wild-type GFP protein from *Renilla reniformis*.

3. A mutant Green Fluorescent Protein (GFP) from *Renilla reniformis*, wherein the mutation comprises an amino acid substitution in the loop region of the protein between Beta Strand 5 and Beta Strand 6, relative to the wild-type form of the protein, and wherein the mutant GFP protein has one or more of the following characteristics:
- (a) enhanced emission intensity, relative to wild-type GFP protein from *Renilla reniformis*;
- (b) a narrower or broader emission spectrum, relative to wild-type GFP protein from *Renilla reniformis*;
- (c) a shift in excitation or emission maxima, relative to wild-type GFP protein from *Renilla reniformis*;
- (d) a shift in maturation rate, relative to wild-type GFP protein from *Renilla reniformis*; and
- (e) exhibiting less quenching of fluorescence at acidic pH, relative to wild-type GFP protein from *Renilla reniformis*.
4. A mutant Green Fluorescent Protein (GFP) from *Renilla reniformis*, wherein the mutation comprises an amino acid substitution in the region of the protein extending from the beginning of Beta Strand 1 through the end of the loop region between Beta Strands 2 and 3, relative to the wild-type form of the protein, and wherein the mutant GFP protein has one or more of the following characteristics:
- (a) enhanced emission intensity, relative to wild-type GFP protein from *Renilla reniformis*;
- (b) a narrower or broader emission spectrum, relative to wild-type GFP protein from *Renilla reniformis*;
- (c) a shift in excitation or emission maxima, relative to wild-type GFP protein from *Renilla reniformis*;
- (d) a shift in maturation rate, relative to wild-type GFP protein from *Renilla reniformis*; and
- (e) exhibiting less quenching of fluorescence at acidic pH, relative to wild-type GFP protein from *Renilla reniformis*.

5. A mutant Green Fluorescent Protein (GFP) from *Renilla reniformis*, wherein the mutation comprises an amino acid substitution in the region of the protein extending from the beginning of Beta Strand 4 through the end of Beta Strand 6, relative to the wild-type form of the protein, and wherein the mutant GFP protein has one or more of the following characteristics:
- (a) enhanced emission intensity, relative to wild-type GFP protein from *Renilla reniformis*;
- (b) a narrower or broader emission spectrum, relative to wild-type GFP protein from *Renilla reniformis*;
- (c) a shift in excitation or emission maxima, relative to wild-type GFP protein from *Renilla reniformis*;
- (d) a shift in maturation rate, relative to wild-type GFP protein from *Renilla reniformis*; and
- (e) exhibiting less quenching of fluorescence at acidic pH, relative to wild-type GFP protein from *Renilla reniformis*.
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6. A polynucleotide encoding mutant *Renilla reniformis* Green Fluorescent Protein (GFP) of claims 1-5.
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7. The polynucleotide of claim 6, said polynucleotide being humanized.
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8. A vector comprising the polynucleotide of claim 7.
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9. A host cell containing the vector of claim 8.
10. A mutant Green Fluorescent Protein (GFP) from *Renilla reniformis*, selected from the group consisting of:
- (a) the amino acid sequence of mutant GM1;
- (b) the amino acid sequence of mutant GM2;
- (c) the amino acid sequence of mutant GM3;
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- (d) the amino acid sequence of mutant GM4;
- (e) the amino acid sequence of mutant GM6;
- (f) the amino acid sequence of mutant T1;
- (g) the amino acid sequence of mutant T6;
- 5 (h) the amino acid sequence of mutant T8;
- (i) the amino acid sequence of mutant T11;
- (j) the amino acid sequence of mutant T12;
- (k) the amino acid sequence of mutant T13;
- (l) the amino acid sequence of mutant T14;
- 10 (m) the amino acid sequence of mutant T15; and
- (n) the amino acid sequence of mutant T17.
11. A polynucleotide encoding a mutant Green Fluorescent Protein (GFP) from *Renilla reniformis*, selected from the group consisting of:
- (a) a polynucleotide encoding the amino acid sequence of mutant GM1;
- (b) a polynucleotide encoding the amino acid sequence of mutant GM2;
- (c) a polynucleotide encoding the amino acid sequence of mutant GM3;
- (d) a polynucleotide encoding the amino acid sequence of mutant GM4;
- (e) a polynucleotide encoding the amino acid sequence of mutant GM6;
- 20 (f) a polynucleotide encoding the amino acid sequence of mutant T1;
- (g) a polynucleotide encoding the amino acid sequence of mutant T6;
- (h) a polynucleotide encoding the amino acid sequence of mutant T8;
- (i) a polynucleotide encoding the amino acid sequence of mutant T11;
- (j) a polynucleotide encoding the amino acid sequence of mutant T12;
- 25 (k) a polynucleotide encoding the amino acid sequence of mutant T13;
- (l) a polynucleotide encoding the amino acid sequence of mutant T14;
- (m) a polynucleotide encoding the amino acid sequence of mutant T15; and
- (n) a polynucleotide encoding the amino acid sequence of mutant T17.
- 30 12. The polynucleotide of claim 11, said polynucleotide being humanized.

13. A vector comprising the polynucleotide of claim 12.
14. A host cell containing the vector of claim 13.
- 5 15. A mutant Green Fluorescent Protein (GFP) from *Renilla reniformis*, selected from the group consisting of:
- 10 (a) the amino acid sequence of SEQ ID NO:34;
- (b) the amino acid sequence of SEQ ID NO:36;
- (c) the amino acid sequence of SEQ ID NO:38;
- (d) the amino acid sequence of SEQ ID NO:40;
- (e) the amino acid sequence of SEQ ID NO:42;
- (f) the amino acid sequence of SEQ ID NO:44;
- (g) the amino acid sequence of SEQ ID NO:46;
- (h) the amino acid sequence of SEQ ID NO:48;
- 15 (i) the amino acid sequence of SEQ ID NO:50;
- (j) the amino acid sequence of SEQ ID NO:52;
- (k) the amino acid sequence of SEQ ID NO:54;
- (l) the amino acid sequence of SEQ ID NO:56;
- (m) the amino acid sequence of SEQ ID NO:58; and
- 20 (n) the amino acid sequence of SEQ ID NO:60.
16. A polynucleotide encoding a mutant Green Fluorescent Protein (GFP) from *Renilla reniformis*, selected from the group consisting of:
- 25 (a) the polynucleotide sequence of SEQ ID NO:33;
- (b) the polynucleotide sequence of SEQ ID NO:35;
- (c) the polynucleotide sequence of SEQ ID NO:37;
- (d) the polynucleotide sequence of SEQ ID NO:39;
- (e) the polynucleotide sequence of SEQ ID NO:41;
- (f) the polynucleotide sequence of SEQ ID NO:43;
- 30 (g) the polynucleotide sequence of SEQ ID NO:45;
- (h) the polynucleotide sequence of SEQ ID NO:47;

- (i) the polynucleotide sequence of SEQ ID NO:49;
(j) the polynucleotide sequence of SEQ ID NO:51;
(k) the polynucleotide sequence of SEQ ID NO:53;
5 (l) the polynucleotide sequence of SEQ ID NO:55;
(m) the polynucleotide sequence of SEQ ID NO:57; and
(n) the polynucleotide sequence of SEQ ID NO:59.
17. The polynucleotide of claim 16, said polynucleotide being humanized.
- 10 18. A vector comprising the polynucleotide of claim 17.
19. A host cell containing the vector of claim 18.
20. A mutant Green Fluorescent Protein (GFP) from *Renilla reniformis*, selected from the
15 group consisting of:
(a) the amino acid sequence of SEQ ID NO:4;
(b) the amino acid sequence of SEQ ID NO:6;
(c) the amino acid sequence of SEQ ID NO:8;
(d) the amino acid sequence of SEQ ID NO:10;
20 (e) the amino acid sequence of SEQ ID NO:12;
(f) the amino acid sequence of SEQ ID NO:14;
(g) the amino acid sequence of SEQ ID NO:16;
(h) the amino acid sequence of SEQ ID NO:18;
(i) the amino acid sequence of SEQ ID NO:20;
25 (j) the amino acid sequence of SEQ ID NO:22;
(k) the amino acid sequence of SEQ ID NO:24;
(l) the amino acid sequence of SEQ ID NO:26;
(m) the amino acid sequence of SEQ ID NO:28; and
(n) the amino acid sequence of SEQ ID NO:30.

21. A polynucleotide encoding a mutant Green Fluorescent Protein (GFP) from *Renilla reniformis*, selected from the group consisting of:
- (a) the polynucleotide sequence of SEQ ID NO:3;
 - (b) the polynucleotide sequence of SEQ ID NO:5;
 - 5 (c) the polynucleotide sequence of SEQ ID NO:7;
 - (d) the polynucleotide sequence of SEQ ID NO:9;
 - (e) the polynucleotide sequence of SEQ ID NO:11;
 - (f) the polynucleotide sequence of SEQ ID NO:13;
 - (g) the polynucleotide sequence of SEQ ID NO:15;
 - 10 (h) the polynucleotide sequence of SEQ ID NO:17;
 - (i) the polynucleotide sequence of SEQ ID NO:19;
 - (j) the polynucleotide sequence of SEQ ID NO:21;
 - (k) the polynucleotide sequence of SEQ ID NO:23;
 - (l) the polynucleotide sequence of SEQ ID NO:25;
 - 15 (m) the polynucleotide sequence of SEQ ID NO:27; and
 - (n) the polynucleotide sequence of SEQ ID NO:29.
22. A vector comprising the polynucleotide of claim 21.
- 20 23. A host cell containing the vector of claim 22.
24. A method of producing mutant *Renilla reniformis* GFP comprising the steps of:
- (a) culturing a cell containing a recombinant vector comprising a wild type or humanized polynucleotide sequence encoding mutant *Renilla reniformis* GFP under conditions where the mutant *Renilla reniformis* GFP protein is expressed; and
 - 25 (b) isolating said mutant *Renilla reniformis* GFP protein from said cell; thereby producing mutant *Renilla reniformis* GFP.
- 30 25. A method of producing a *Renilla reniformis* fusion protein, said method comprising the steps of: culturing a cell containing a polynucleotide sequence encoding said polypeptide

of interest linked with a humanized polynucleotide encoding mutant *Renilla reniformis* GFP wherein the linked polynucleotide sequences are fused in frame, under conditions where the mutant *Renilla reniformis* GFP protein is expressed.

- 5 26. A method of determining the location of a polypeptide of interest in a cell, said method comprising determining the location of the fusion protein of claim 25.
27. A method of identifying a cell into which a recombinant vector has been introduced, said method comprising the steps of:
- 10 (a) providing a cell containing a recombinant vector comprising a humanized polynucleotide which encodes mutant *Renilla reniformis* GFP, wherein said cell permits expression of said humanized polynucleotide;
- 15 (b) illuminating said population with light within the excitation spectrum of mutant *Renilla reniformis* GFP; and
- 15 (c) detecting fluorescence in the emission spectrum of mutant *Renilla reniformis* GFP in said population, where detection of fluorescence in the cell indicates that the recombinant vector has been introduced into the cell;
thereby identifying a cell into which said recombinant vector has been introduced.
- 20 28. The method of claim 27, wherein said GFP is expressed as a fusion polypeptide.
29. The method of claim 27, wherein said GFP is expressed as a distinct polypeptide.
30. The method of claim 27, wherein said cells are identified by FACS analysis.
- 25 31. A method of detecting the activity of a transcriptional regulatory sequence, said method comprising the steps of:
- 30 (a) culturing a cell containing a nucleic acid sequence comprising said transcriptional regulatory sequence operably linked to a humanized nucleic acid sequence encoding mutant *Renilla reniformis* GFP to form a reporter construct, under conditions where the mutant *Renilla reniformis* GFP is expressed; and

- (b) detecting mutant *Renilla reniformis* GFP fluorescence in said cell, wherein detection of fluorescence indicates activity of said transcriptional regulatory sequence;

thereby detecting the activity of a transcriptional regulatory sequence.

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32. A method of detecting the presence of a modulator of a transcriptional regulatory sequence, said method comprising the steps of:
- (a) culturing a cell containing a nucleic acid sequence comprising said transcriptional regulatory sequence operably linked to a humanized nucleic acid sequence encoding mutant *Renilla reniformis* GFP to form a reporter construct, under conditions where the mutant *Renilla reniformis* GFP is expressed; and
 - (b) detecting mutant *Renilla reniformis* GFP fluorescence in said cell, wherein said fluorescence indicates the presence of said modulator;
- thereby detecting the presence of a modulator of a transcriptional regulatory sequence.
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33. A method of screening for an inhibitor of a transcriptional regulatory sequence, said method comprising the steps of:
- (a) culturing a cell containing a nucleic acid sequence comprising said transcriptional regulatory sequence operably linked to a humanized nucleic acid sequence encoding mutant *Renilla reniformis* GFP to form a reporter construct, under conditions where the mutant *Renilla reniformis* GFP is expressed;
 - (b) contacting said cell with a candidate inhibitor of said transcriptional regulatory sequence; and
 - (c) detecting mutant *Renilla reniformis* GFP fluorescence in said cell, wherein a decrease in said fluorescence relative to that detected in the absence of said candidate inhibitor indicates that said candidate inhibitor inhibits the activity of said transcriptional regulatory sequence.
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34. A method of producing a fluorescent molecular weight marker, said method comprising the steps of:
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- (a) culturing a cell containing a humanized nucleic acid sequence encoding mutant *Renilla reniformis* GFP linked in frame to a nucleic acid sequence encoding a polypeptide of known relative molecular weight such that said linked molecules encode a fusion polypeptide, under conditions where the mutant *Renilla reniformis* GFP is expressed;
- (b) isolating said fusion polypeptide from said cell, wherein said fusion polypeptide is a relative molecular weight marker.
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35. The method of claims 24, 25, 27 or 31-34, wherein said cell is a mammalian cell.
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36. The method of claims 24, 25, 27 or 31-34, wherein said cell is a human cell.
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37. The method of claims 24, 25, 27 or 31-34, wherein said mutant *Renilla reniformis* GFP is selected from the group consisting of: SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28 and SEQ ID NO:30.
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38. The method of claims 24, 25, 27 or 31-34, wherein said nucleic acid sequence encoding mutant *Renilla reniformis* GFP is selected from the group consisting of: SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27 and SEQ ID NO:29.
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39. A mutant Green Fluorescent Protein (GFP) from *Renilla reniformis*, wherein the mutation comprises an amino acid substitution in one of the following regions of the protein, relative to the wild-type form of the protein:
- (a) the Beta Strand 4 region of the protein;
- (b) the loop region of the protein between Beta Strand 2 and Beta Strand 3;
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- (c) the loop region of the protein between Beta Strand 5 and Beta Strand 6;

- (d) the region of the protein extending from the beginning of Beta Strand 1 through the end of the loop region between Beta Strands 2 and 3; and
- (e) the region of the protein extending from the beginning of Beta Strand 4 through the end of Beta Strand 6;
- 5 and wherein the mutant GFP protein has one or more of the following characteristics:
- (r) exhibiting less quenching over a broad pH range, relative to wild-type GFP protein from *Renilla reniformis*.;
- (s) exhibiting greater resistance to one or more of the following: proteases, solvents, detergents and chaotropic agents; and
- 10 (t) exhibiting reduced tendency to oligomerize.
40. A mutant Green Fluorescent Protein (GFP) from *Renilla reniformis*, wherein the mutation comprises an amino acid substitution at one or more of the following residues:
- (a) F43;
- 15 (b) E120;
- (c) L101; and
- (d) Y103.